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FORMATION OF 8-OXO-7,8-DIHYDRO-2'-DEOXYGUANOSINE UNDER ANAEROBIC CONDITIONS BY REDUCTIVELY ACTIVATED NITRO 5-DEAZAFLAVIN DERIVATIVES

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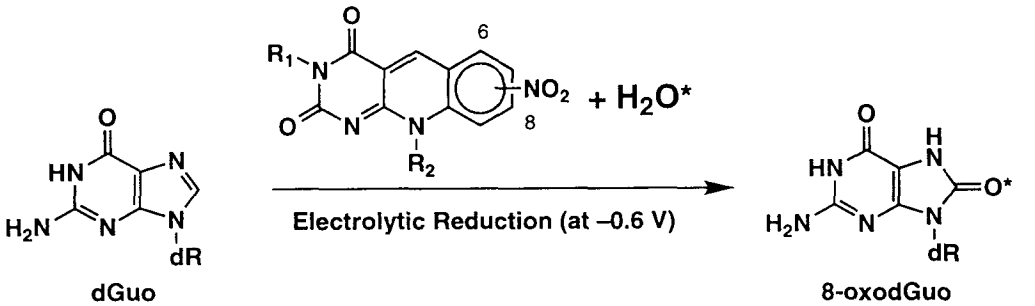
Abstract: Electrolytically reduced 6- and 8-nitro-5-deazaflavin derivatives have been found to interact to react specifically with guanine base by means of cyclic voltammetry. Electrolytic reductions of 6- and 8-nitro-5-deazaflavin derivatives in the presence of the 2'-deoxyguanosine under anaerobic conditions resulted in prominent formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine.

8-Oxo-7,8-dihydro-2'-deoxyguanosine (8-hydroxy-2'-deoxyguanosine, 8-oxodGuo)[2] (Scheme 1) is one of the major products of oxidative DNA damage and has attracted considerable attention as a consequence of its capacity to induce mispairing and its mutagenic abilities.[3] Formation of 8-oxodGuo caused by ROS,[2,4] photosensitizers,[5] and some carcinogens[6] has been studied well, however, its formation by reductively activated nitrohetero-aromatic compounds under anaerobic conditions has not been reported.

Recently, we have developed nitro 5-deazaflavins[7] (Scheme 1) as novel class of nitrohetero-aromatic compounds containing an electrophilic redox coenzyme ring system and showed their marked selective toxicities towards hypoxic cells[7b] as well as DNA damaging effects[7c] under anaerobic conditions. Since the nitro 5-deazaflavin derivatives have higher one-electron reduction potentials[7a,b] than other nitrohetero-aromatic compounds, they would be anticipated to be readily activated by intracellular reductases to give rise to greater oxidative DNA damage under anaerobic conditions.[7c] In the course of our study of mechanisms for their biological actions, we have found that reductively activated nitro 5-deazaflavin derivatives interact to react specifically with guanine moiety, resulting in prominent formation of 8-oxodGuo. In the present paper, we wish to describe the formation of 8-oxodGuo by reductively activated nitro 5-deazaflavin derivatives under anaerobic conditions.



Scheme 1.

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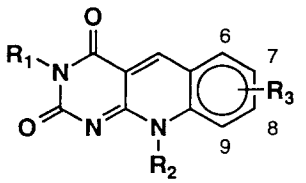
Compounds **1-5** (Scheme 2) were synthesized according to Yoneda's method.[7a,c] Condensation reactions of 6-butylaminouracil or 6-butylamino-3-methyluracil with 2-fluoro-6-nitrobenzaldehyde, 2-fluoro-4-nitrobenzaldehyde, or 2-fluorobenzaldehyde in DMF afforded **1-5** respectively in 70-88%.



1; $R_1 = CH_3$, $R_2 = n\text{-}C_8H_{17}$, $R_3 = 6\text{-}NO_2$
2; $R_1 = CH_3$, $R_2 = n\text{-}C_8H_{17}$, $R_3 = 8\text{-}NO_2$
3; $R_1 = H$, $R_2 = n\text{-}C_4H_9$, $R_3 = 6\text{-}NO_2$
4; $R_1 = H$, $R_2 = n\text{-}C_4H_9$, $R_3 = 8\text{-}NO_2$
5; $R_1 = H$, $R_2 = n\text{-}C_4H_9$, $R_3 = H$

Scheme 2.

Interactions of reductively activated nitro 5-deazaflavin derivatives with 2'-deoxyribonucleosides were investigated by means of cyclic voltammetry (CV).[8] As Figure 1a shows, 6-nitro-5-deazaflavin **1** (Scheme 2) in DMF affords two reversible reduction processes (Ep = –0.546 V and –0.892 V vs Ag/AgCl), suggesting that **1** generates stable one- and two-electron reduction products.[7b] Prominent changes in the CV behavior of **1** corresponding to one-electron redox couple were observed in the presence of 2'-deoxyguanosine (dGuo) (Figure 1b), showing that chemically irreversible processes[9] are growing as the concentration of dGuo increases. The similar CV changes have also been found in the presence of guanosine and 9-ethylguanine. No significant CV changes in one-electron redox couple were found in the presence of 2'-deoxyadenosine (dAdo), thymidine (dThd) and 2'-deoxycytidine (dCyd). The above experimental results were similarly obtained in 8-nitro-5-deazaflavin **2** (Scheme 2). These results suggest that the one-electron reduction product(s) of **1** and **2** interact to react specifically with guanine moiety.

To elucidate the reaction of the reduced nitro 5-deazaflavin derivatives with guanine moiety, electrolytic reductions[7c,10] of 6-nitro-5-deazaflavin **3** and 8-nitro-5-deazaflavin **4** (Scheme 2, 1.0 mM), more hydrophilic compounds[11] in comparison with **1** and **2**, in the presence of nucleosides (0.1 mM) were carried out at a controlled potential of –600 mV vs Ag/AgCl in DMF and in DMF containing 1.5 mM sodium citrate buffer solutions under argon stream at 298 K. The reactions were monitored by means of an HPLC method coupled to UV detector (at 256 nm) and ECD (at 600 mV).[12] As Figure 2 shows, no significant degradation of dAdo,

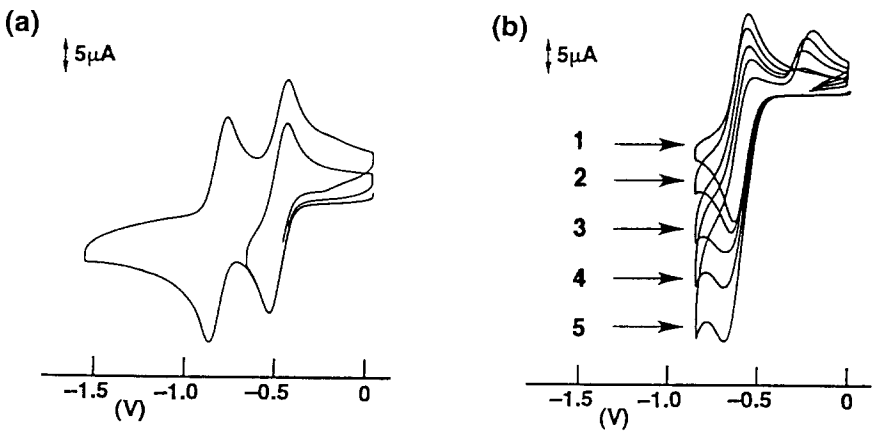


Figure 1. Cyclic voltammograms of compound **1** (a) and compound **1** in the presence of dGuo (b) (at 298K, in DMF, under N_2, [**1**] = 1.0×10^{-3} (M), $[Bu_4NClO_4] = 1.0 \times 10^{-1}$ (M), [dGuo] = (1) 0, (2) 1.0×10^{-3}, (3) 3.0×10^{-3}, (4) 5.0×10^{-3}, (5) 1.0×10^{-2} (M), versus an aqueous Ag/AgCl reference electrode, scan rate $v = 100\ mVs^{-1}$).

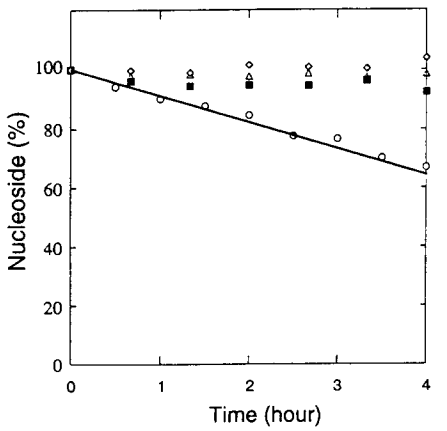


Figure 2. Degradation of 2'-deoxyribonucleosides (dGuo, circle; dAdo, square; dCyd, triangle; dThd, rhombus) induced by electrolytic reduction of **3**
(at 298K, under Ar, in 25% DMF containing 1.5 mM sodium citrate buffer , [**3**] = 1.0 x 10^{-3} (M), [Nucleoside] = 1.0 x 10^{-4} (M)).

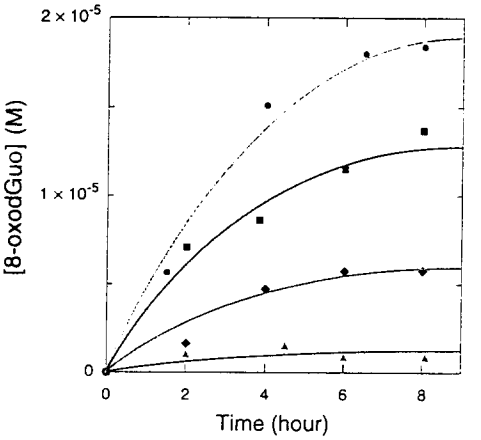


Figure 3. Formation of 8-oxodGuo by electrolytic reduction of **3** in the presence of dGuo in DMF (triangle) and DMF (25%, circle; 50%, square; 75%, rhombus) containing 1.5 mM sodium citrate buffer solutions
(at 298K, under Ar, [**3**] = 1.0 x 10^{-3} (M), [dGuo] = 1.0 x 10^{-4} (M)).

dThd, and dCyd was found. Interestingly enough, significant formation of 8-oxodGuo concomitant with degradation of dGuo has been observed as the reduction of **3** and **4** proceeded (Figure 2 and 3).[13] Electrolysis of the dGuo solutions at a controlled potential of –600 mV in the absence of **3** and **4**, and electrolysis in the presence of a 5-deazaflavin derivative **5** (Scheme 2) with no nitro group has been found to induce essentially no formation of 8-oxodGuo nor degradation of dGuo.[14] Furthermore, an addition of dGuo to the reaction solution after completion of electrolytic reductions of **3** and **4** induced no significant modification of dGuo. These results suggest that reduction intermediate(s) of **3** and **4** interact to react specifically with dGuo giving rise to formation of 8-oxodGuo.

It is worthy of notice that the formation of 8-oxodGuo has been found to be enhanced as the water content of the employed reaction solution increases. When 1.0 x 10^{-3} (M) of **3** was subjected to electrolytic reduction in the presence of 1.0 x 10^{-4} (M) of dGuo in 25 % DMF containing sodium citrate buffer for 8 hours, formation of about 2 x 10^{-5} (M) of 8-oxodGuo was observed as major product concomitant with degradation of 40% of dGuo employed (Figure 3). Essentially no formation of 2,4-diamino-5-formamidopyrimid-6-ones (FapyGua and its derivatives)[15] was found by means of HPLC analyses[15,16,17] in the present reaction system. In order to investigate the reaction mechanism for formation of 8-oxodGuo, electrolysis of **3** and **4** in the presence of dGuo in the reaction solution containing 95% [^{18}O]H_2O (50% DMF containing 1.5 mM sodium citrate buffer) was carried out under anaerobic condition. GC-MS analysis of tetrakis(trimethylsilyl) derivative[18] of 8-oxo-7,8-dihydroguanine gave a molecular ion peak of m/z 457 (M^+) concomitant with a peak of m/z 442 (M^+–CH_3) which corresponds to the molecular weight of [^{18}O]-8-oxo-7,8-dihydroguanine, indicating an incorporation of an ^{18}O atom from the solvent into the guanine moiety (Scheme 1).

The above experimental results imply that reduced 6- and 8-nitro-5-deazaflavin derivatives could induce oxidative DNA damage[19] at dGuo to generate 8-oxodGuo under hypoxic conditions, which would lead to their selective cytotoxicities towards hypoxic cells. In conclusion, we have first demonstrated that reductively activated 6- and 8-nitro-5-deazaflavin derivatives interact to react specifically with guanine moiety, giving rise

to formation of 8-oxodGuo. The detailed reaction mechanism for formation of 8-oxodGuo by reductively activated 6- and 8-nitro-5-deazaflavin derivatives is under investigation.

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References and Notes

1. Present address: Fujimoto Pharmaceutical Co., Ltd. Matsubara, Osaka 580-0011, Japan.
2. Kasai, H.; Nishimura, S. *Nucleic Acids Res.* **1984**, *12*, 2137-2145.
3. a) Shibutani, S.; Takeshita, M.; Grollman, A. P. *Nature* **1991**, *349*, 431-434. b) Wood, M. L.; Dizdaroglu, M.; Gajewski, E.; Essigmann, J. M. *Biochemistry* **1990**, *29*, 7024-7032. c) Le Page F.; Guy, A.; Cadet, J.; Sarasin, A.; Gentil, A. *Nucleic Acids Res.* **1998**, *26*, 1276-1281.
4. a) Cadet, J.; Teoule, R. *Photochem. Photobiol.* **1978**, *28*, 661-667. b) Wei, H.; Cai, Q.; Rahn, R.; Zhang, X. *Free Rad. Biol. Med.* **1997**, *23*, 148-154.
5. a) Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1992**, *114*, 9692-9694. b) Buchko, G. W.; Cadet, J.; Berger, M.; Ravanat, J.-L. *Nucleic Acids Res.* **1992**, *20*, 4847-4851.
6. a) Fiala, E. S.; Conaway, C. C.; Mathis, J. E. *Cancer Res.* **1989**, *49*, 5518-5522. b) Kohda, K.; Tada, M.; Kasai, H.; Nishimura, S.; Kawazoe, Y. *Biochem. Biophys. Res. Comms.* **1986**, *139*, 626-632.
7. a) Kawamoto, T.; Ikeuchi, Y.; Hiraki, J.; Eikyu, Y.; Shimizu, K.; Tomishima, M.; Bessho, K.; Yoneda, F.; Mikata, Y.; Nishida, M.; Ikehara, K.; Sasaki, T. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2109-2114. b) Kawamoto, T.; Ikeuchi, Y.; Hiraki, J.; Eikyu, Y.; Shimizu, K.; Tomishima, M.; Bessho, K.; Yoneda, F.; Mikata, Y.; Nishida, M.; Ikehara, K.; Sasaki, T. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2115-2118. c) Kanaoka, Y.; Ikeuchi, Y.; Kawamoto, T.; Bessho, K.; Akimoto, N.; Mikata, Y.; Nishida, M.; Yano, S.; Sasaki, T.; Yoneda, F. *Bioorg. Med. Chem.* **1998**, *6*, 301-314.
8. a) Tocher, J. H.; Edwards, D. I. *Int. J. Radiation Oncology Biol. Phys.* **1992**, *22*, 661-663. b) Tocher, J. H.; Edwards, D. I. *Biochem. Pharmacol.* **1994**, *48*, 1089-1094.
9. Increase in the reduction peak current and appearance of a new oxidation wave may suggest the reaction product of **1** and **2** would undergo further redox process.
10. a) Edwards, D. I. *Biochem. Pharmacol.* **1986**, *35*, 53-58. b) A. Zahoor, A.; Lafleur, M.V.M.; Knight, R. C.; Loman, H.; Edwards, D. I. *Biochem. Pharmacol.* **1987**, *36*, 3299-3304. c) Declerck, P. J.; De Ranter, C, J.; Volckaert, G. *FEBS Lett.* **1983**, *164*, 145-148.
11. Because of the limited solubility of **3** and **4** in DMF, compounds **1** and **2** were employed for CV measurements in DMF. The substituents at N(3) and N(10) position, R_1 and R_2, affect essentially the solubility of the compounds in the employed solvent and give no significant effects on their redox properties.
12. Rosier, J. A.; van Peteghem, C. H. *J. Liq. Chromatogr.* **1988**, *11*, 1293-1298. Authentic 8-oxodGuo (8-oxodGuo•H_2O) was purchased from Cayman Chemical Company.
13. In separate experiments, electrolytic reductions of **3** and **4** (1.0×10^{-3} (M)) at –600 mV in the presence of 8-oxodGuo (1.0×10^{-5} (M)) in 25% DMF containing 1.5 mM sodium citrate buffer solution for 4 hours resulted in about 30% degradation of 8-oxodGuo. The results suggest that competitive oxidation of 8-oxodGuo is accompanied with its generation from dGuo by reductively activated **3** and **4** and the yield of 8-oxodGuo from dGuo would be higher than is estimated from the results shown in Figure 2 and 3.
14. Electrolysis of dGuo solution (25% DMF containing 1.5 mM sodium citrate buffer) at –600 mV under aerobic condition for 4 hours resulted in formation of 1.0×10^{-5} (M) of 8-oxodGuo. Electrolytic reduction of 7- and 9-nitro-5-deazaflavin derivatives in the presence of dGuo gave essentially no formation of 8-oxodGuo nor degradation of dGuo.
15. Berger, M.; Cadet, J. *Z. Naturforsch.* **1985**, *40b*, 1519-1531.
16. Kaur, H.; Halliwell, B. *Biochem. J.* **1996**, *318*, 21-23.
17. The results suggest that 8-oxodGuo would not be generated via 8-hydroxy-7,8-dihydroguanyl radical (or its related opened imidazole ring form) as a reaction intermediate in the present reaction system. The detailed reaction mechanism is under investigation.
18. a) Dizdaroglu, M. *Methods Enzymol.* **1994**, *234*, 3-16; b) Dizdaroglu, M. *Free Radical Biol. Med.* **1991**, *10*, 225-242.
19. In separate experiments, electrolytic reductions of 6- and 8-nitro-5-deazaflavin derivatives (1.0 mM) in the presence of calf-thymus DNA (500 μM/base) in sodium citrate buffer under argon stream for 8 hours resulted in formation of 5.0×10^{-6} (M) of 8-oxodGuo.